Effect of graded levels of iron, zinc, and copper supplementation in diets with low-phytate or normal barley on growth performance, bone characteristics, hematocrit volume, and zinc and copper balance of young swine[1]

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ABSTRACT: Fifty crossbred barrows with an average initial age of 31 d and BW of 9.94 kg were used in a 28-d experiment to evaluate the effect of a low-phytic acid (LPA) barley mutant (M) M955, a near-isogenic progeny of the normal barley (NB) cultivar Harrington with about 90% less phytate than NB, to increase the utilization of Fe, Zn, and Cu compared with diets containing NB. The response criteria were growth performance, hematocrit volume, metacarpal bone characteristics, and the apparent absorption, retention, and excretion of Zn and Cu. The 2 barley cultivars (NB and M955) and the 5 trace mineral (TM) treatment concentrations of Fe and Zn (0, 25, 50, 75, and 100% of the requirement as $FeSO_4$ and $ZnSO_4$) and Cu (0, 40, 80, 120, and 160% of the requirement as $CuSO_4$) made 10 treatments in a factorial arrangement. Available P was equalized at 0.33% in all diets by adding monosodium phosphate to the basal diet containing NB, and all diets contained 0.65% Ca. Diets were adequate in all other nutrients. Barley and soybean meal were the only sources of phytate in the practical diets that also contained spray-dried whey. The barrows were fed the diets to appetite in meal form twice daily in individual metabolism crates. There were no barley cultivar × TM treatment interactions, and there were no differences between the NB and M955 barley cultivars for any of the response criteria measured. However, for the TM treatments, there were linear increases ($P \leq 0.05$) in ADFI, ADG, hematocrit volume, metacarpal bone breaking strength and ash weight, and the apparent absorption, retention, and excretion (mg/d) of Zn and Cu. In conclusion, the LPA barley had no effect on the response criteria in this experiment, apparently because of the small increase in the availability of the endogenous trace minerals in the practical diets containing M955 compared with NB. However, increasing the supplementation of Fe and Zn from 0 to 100% (160% for Cu) of the requirement resulted in linear increases in growth performance, hematocrit volume, metacarpal bone strength and ash weight, and the apparent absorption, retention, and excretion of Zn and Cu. Therefore, these results indicate that the inorganic trace mineral supplementation of practical diets for young pigs should not be less than the National Research Council requirements for swine.

Key words: barley, growth performance, nutrient balance, phytate, swine, trace mineral

J. Anim. Sci. 2009. 87:2625–2634
doi:10.2527/jas.2008-1604

INTRODUCTION

The endogenous minerals in grain-soybean meal (**SBM**) diets fed to swine are poorly utilized because swine produce little or no intestinal phytase (Pointillart et al., 1984, 1987). Consequently, most of the phytate P in grain-SBM diets is excreted in the pig manure (Veum et al., 2001, 2002, 2007). The small intestine is the principal site of mineral absorption in swine (Partridge, 1978; Liu et al., 2000). Some microbial hydrolysis of phytate occurs in the hindgut of the pig (Leytem et al., 2004; Angel et al., 2005), and variable amounts of P and Ca are absorbed from the cecum and colon (Liu et al., 2000). Phosphorus absorption was increased and P excretion reduced by replacing some or all of the inorganic P supplement with a phytase enzyme product in corn-SBM diets fed to growing or finishing

[1]Supported in part by the Missouri Agric. Exp. Sta., the USDA-ARS Small Grains and Potato Germplasm Research Unit, Aberdeen, ID, and the Idaho Barley Commission, Boise, ID. We thank D. Bollinger, J. Smith, A. Tsunoda, C. Wu, M. Gore, and K. Ingram (University of Missouri, Columbia) for assistance with animal care and data collection.

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Received November 3, 2008.
Accepted April 6, 2009.

swine (Liu et al., 1998; Veum et al., 2006; Veum and Ellersieck, 2008). In addition, phytase supplementation increased the trace element utilization in grain-SBM diets for weanling pigs (Lei et al., 1993; Kirchgessner et al., 1994; Adeola et al., 1995) and for growing and finishing pigs (Peter et al., 2001; Masuda et al., 2002).

The development of low-phytic acid (**LPA**) grains (Larson et al., 1998; Raboy et al., 2001; Dorsch et al., 2003) creates the potential to increase mineral utilization and reduce mineral excretion by nonruminant animals globally (Veum et al., 2002, 2007; Linares et al., 2007). Experiments have shown that LPA grains are effective in increasing the absorption and reducing the excretion of Ca and P by growing swine (Veum et al., 2001, 2002, 2007). The objective of this experiment was to evaluate the efficacy of a LPA barley mutant (**M**) cultivar M955 compared with the corresponding normal barley (**NB**) cultivar to increase the utilization of Fe, Zn, and Cu in barley-SBM diets fed to growing swine. The response criteria were pig growth performance, hematocrit volume, metacarpal bone characteristics, and the apparent absorption, retention, and excretion of Zn and Cu.

MATERIALS AND METHODS

The procedures and use of animals in this experiment were approved by the University of Missouri Animal Care and Use Committee.

Animals and Housing

Crossbred pigs (GenetiPorc US, LLC, Morris, MN) were injected with 100 mg of Fe as iron dextran within 3 d of birth, weaned at 17 ± 1 d of age, and fed a phase 1 nursery diet for a 2-wk acclimation period before beginning this 28-d experiment. The acclimation diet contained 40.5% ground yellow corn, 25.0% spray-dried whole whey, 18.0% dehulled SBM, 7.0% spray-dried animal plasma, 4.0% corn oil, 1.75% spray-dried blood cells, and mineral and vitamin supplementation to meet or exceed the NRC (1998) requirements for 5- to 10-kg swine. The trace mineral premix in the acclimation diet provided 165 mg of Fe as $FeSO_4$, 165 mg of Zn as $ZnSO_4$, 16.5 mg of Cu as $CuSO_4$, 33.0 mg of Mn as $MnSO_4$, 0.3 mg of I as $Ca(IO_3)_2$, and 0.285 mg of Se as Na_2SeO_2 per kilogram of diet.

Fifty barrows with an average BW of 9.94 ± 0.40 kg and 31 ± 1 d of age were allotted to 10 dietary treatments by litter and BW in a completely random design. The barrows were placed in individual, elevated, solid-walled, stainless-steel metabolism crates (floor space: 1 replication with 0.7 m^2, 3 replications with 0.9 m^2, and 1 replication with 1.3 m^2/pig) equipped with stainless-steel drinkers, feeders, and woven wire or slotted flooring, with 5 replications/treatment. A room temperature of about 25°C (range of 24.4 to 25.6°C) was maintained during wk 1 with heaters and exhaust fans, and was reduced 1°C each week thereafter, with 15 h of light beginning at 0600 h. The barrows were fed the air-dry diets in meal form to appetite twice daily (0730 and 1600 h).

Barley Cultivars and Dietary Treatments

The wild-type NB and the LPA cultivar M955 used in this experiment were produced at the Small Grains and Potato Germplasm Research Unit (Aberdeen, ID) and transported to the University of Missouri. The NB was the cultivar Harrington, a hulled 2-row malting barley that was homozygous for the wild-type alleles of the phytic acid genes, and produced grain with a normal concentration of phytic acid (Table 1). The M955 cultivar was isolated from the cultivar Harrington (Dorsch et al., 2003) and is a near-isogenic LPA variant of the Harrington cultivar. A previous experiment reported that the M955 barley cultivar improved the utilization of P, Ca, N, energy, and DM compared with NB in diets fed to young pigs (Veum et al., 2007).

The barley cultivars and SBM were the only sources of phytic acid in the 2 basal barley cultivar diets (Table 2). Available P (**aP**) was calculated by subtracting the analyzed concentration of phytic acid P from the analyzed concentration of total P (**tP**). Protein ingredient supplementation was equalized for both basal barley cultivar diets to standardize the biological value of the supplemental protein, the addition of endogenous trace elements from the protein sources, and the addition of phytate from SBM. Available P was equalized at 0.33% in all the diets containing NB or M955 by adding monosodium phosphate to the diets containing NB. Monosodium phosphate was used because it contains less Fe than dicalcium phosphate (NRC, 1998). Calcium was equalized at 0.65% in all diets using ground limestone, with a Ca:aP ratio of 1.97. Crystalline AA supplementation was used to meet the essential AA requirements. Dietary ME, calculated by using ingredient values for ME from NRC (1998), was about 3.3 Mcal/kg for both basal barley cultivar diets. An enzyme product (Ronozyme B, Hoffmann La-Roche Inc., Nutley, NJ) was added to the basal barley cultivar batch mixes to prevent problems with the nonstarch polysaccharides in barley and provided per kilogram of diet: 70 U of endo-1,3(4)-β-glucanase (1 U = the amount of enzyme that releases 1.0 μM of glucose or reducing carbohydrate/min at pH 5.0 and 30°C), 300 U of endo-1,4-β-xylanase (1 U = the amount of enzyme that releases 7.8 μM of reducing xylose equivalents from azo-wheat arabinoxylan/min at pH 6.0 and 50°C), and 25 U of α-amylase (1 U = the amount of enzyme that breaks down 5.26 g of starch/h at pH 7.1 and 37°C).

The 2 barley cultivars (NB and M955) and 5 concentrations of the trace minerals Fe, Zn, and Cu made 10 treatments in a 2 × 5 factorial arrangement. The 5 trace mineral (**TM**) treatments provided increasing concentrations of Fe, Zn, and Cu as $FeSO_4$, $ZnSO_4$, and $CuSO_4$, respectively, per kilogram of diet: **TM0** = no supplemental Fe, Zn, or Cu; **TM25** = 20 mg each of

Table 1. Analyzed chemical composition (%) of air-dried barley cultivars, soybean meal, and spray-dried whey, as-fed basis[1]

Item	Low-phytate mutant 955 barley	Wild type normal barley	Soybean meal	Spray-dried whey
DM	88.20	88.10	87.94	96.00
Ash	2.33	2.30	6.45	7.69
Ca	0.06	0.06	0.34	0.74
Total P	0.35	0.36	0.70	0.70
Phytate P	0.025	0.24	0.43	—
Available P[2]	0.325	0.12	0.27	0.70
Fe, mg/kg	67.9	89.7	203.1	56.5
Zn, mg/kg	25.4	23.8	60.4	9.9
Cu, mg/kg	6.2	5.5	16.8	6.6
Crude fat	1.32	1.72	1.28	0.80
Crude fiber	3.74	3.17	3.51	0.11
CP	11.63	10.46	47.76	12.50
Lys	0.43	0.38	3.01	0.92
Met	0.20	0.18	0.71	0.17
Cys	0.27	0.24	0.81	0.27
Trp	0.12	0.10	0.66	0.19
Thr	0.37	0.32	1.84	0.73
Ile	0.37	0.33	2.15	0.67
Val	0.52	0.47	2.25	0.64

[1]Average of triplicate samples of ingredients.
[2]Available P was calculated by subtracting phytate P from total P.

Fe and Zn, and 2 mg of Cu; **TM50** = 40 mg each of Fe and Zn, and 4 mg of Cu; **TM75** = 60 mg each of Fe and Zn, and 6 mg of Cu; and **TM100** = 80 mg each of Fe and Zn, and 8 mg of Cu. The TM0, TM25, TM50, TM75, and TM100 treatments provided 0, 25, 50, 75, and 100%, respectively, of the requirement for Fe and Zn; and 0, 40, 80, 120, and 160%, respectively, of the requirement for Cu for 10- to 20-kg of BW pigs (NRC, 1998). Also, all the TM treatments provided 16 mg of Mn as $MnSO_4$, 0.15 mg of I as $Ca(IO_3)_2$, and 0.25 mg of Se as Na_2SeO_2 per kilogram of diet. Our standard swine TM premix was used to provide the 5 graded concentrations of Fe, Zn, and Cu for the TM treatments, and the concentrations of Mn, I, and Se were brought up to 100% of the requirement for each TM treatment with additional supplementation. There was no depletion feeding period for Fe, Zn, and Cu before starting this experiment. Therefore, treatment TM0 is equivalent to a 28-d withdrawal of Fe, Zn, and Cu when evaluating the LPA effect of the M955 barley vs. the NB cultivar on the TM treatment response criteria.

Measurements

Barley Cultivar and Diet Analysis. Before formulation of the NB and the M955 barley cultivar basal diets, stocks of the NB and M955 barley cultivars, SBM, and spray-dried whey were sampled (triplicate samples used for all analyses) and analyzed for proximate analysis components (AOAC, 1990), GE by oxygen bomb calorimetry (Parr Instrument Co., Moline, IL), and complete AA (Benson and Patterson, 1971), with the analyzed values presented in Table 1. The samples were hydrolyzed under N with 6 *N* HCl for 24 h at 110°C before AA analysis was performed by cation-exchange chromatography. Analyses for cystine and methionine involved performic acid oxidation before hydrolysis. Tryptophan was determined by the method of Spies and Chambers (1949). Samples of the above ingredients plus monosodium phosphate and ground limestone were digested using a wet ash procedure (AOAC, 1990), except that the samples were ashed at 540°C to prevent Zn volatilization. The digests were analyzed for the concentrations of tP by the molybdovanadate colorimetric method (Spectra Rainbow Microplate Reader, Tecan Inc., Durham, NC), and for the concentrations of Ca, Fe, Zn, and Cu by atomic absorption spectrophotometry (Spector AA-30, Varian Analytical Instruments, San Fernando, CA). The standard swine TM premix used was also analyzed for Fe, Zn, and Cu to confirm the guaranteed concentrations (Nutra Blend Corporation, Neosho, MO). Samples of the barley cultivars and SBM were analyzed for phytate as described by Raboy et al. (2000). The analyzed nutrient values of the ingredients were used to formulate the NB and M955 barley cultivar basal diet batch mixes (Table 2). After mixing, the diets were sampled and analyzed for Zn, Cu, and Cr (AOAC, 1990), and the analyzed values were used to determine Zn and Cu balance.

Animal, Blood, and Bone Measurements. Feed consumption of individual barrows was determined weekly and from d 22 to 26 to determine the apparent nutrient balance of Zn and Cu. The barrows were weighed on d 0, 14, and 28 (the end) of the experiment. On d 13 and 27, blood samples (5 to 6 mL) were collected by vena cava puncture from individual pigs into 10-mL evacuated tubes containing 143 units of sodium heparin per tube for the determination of hematocrit volume (McGovern et al., 1955). On d 28, barrows were killed (stunned by captive bolt followed by exsanguination). The right front foot of each pig was removed and stored at 2°C in a plastic bag. The third metacarpal bone was excised and cleaned of all adhering tissue within 3 d for bone size and weight measurements, and for the determination of breaking strength and ash weight. A caliper (model CDS6, Mitutoyo Corp., Kawasaki, Japan) was used to measure the metacarpal bone length and midshaft widths at the narrowest and widest points. Breaking strength of the fresh bones was determined using an Instron testing machine (model TML, Instron Corp., Canton, MA), similar to the procedure described by Crenshaw (1986). Force was applied to the center of the bone, which was held by 2 supports spaced 3.0 cm apart. After determination of the breaking strength, the bones were wrapped in cheesecloth, boiled in deionized water for 2 h, dried at 55°C for 24 h, and extracted with ethyl ether for 4 d. Ash weight was determined after the fat-free bones were dried at 55 and 100°C for 18 and 2 h, respectively, and ashed in a muffle furnace at 540°C for 24 h (AOAC, 1990).

Table 2. Ingredient and chemical composition (%) of air-dried basal diets, as-fed basis

	Barley cultivars	
Item	Low-phytate mutant 955	Wild type normal
Ingredient		
Barley cultivar	52.75	51.98
Soybean meal, 48% CP	23.50	23.50
Spray-dried whey	15.00	15.00
Lard	6.30	6.54
Ground limestone	0.98	0.98
Salt, noniodized	0.40	0.40
Vitamin and enzyme premixes[1]	0.40	0.40
Monosodium phosphate[2]	—	0.43
L-Lys·HCl	0.20	0.24
DL-Met	0.05	0.08
L-Thr	0.05	0.08
Tylan[3]	0.12	0.12
Chromic oxide	0.05	0.05
Trace mineral premix[4]	0.20	0.20
Chemical composition[5]		
Ca	0.65	0.65
Total P	0.46	0.56
Phytic acid P	0.13	0.23
Available P	0.33	0.33
Fe, mg/kg	92.1	102.9
Zn, mg/kg	29.1	28.1
Cu, mg/kg	8.2	7.8
CP	19.20	18.50
Lys	1.23	1.23
Met + Cys	0.73	0.75
Thr	0.78	0.78
Trp	0.25	0.24
ME, Mcal/kg	3.31	3.31

[1]Vitamin premix provided per kilogram of diet: 11,000 IU of vitamin A acetate; 1,100 IU of vitamin D_3; 4.4 IU of vitamin E from DL-α-tocopheryl acetate; 4.0 mg of vitamin K from menadione sodium dimethylprimidinol bisulfite; 30.3 μg of vitamin B_{12}; 8.3 mg of riboflavin; 28.1 mg of pantothenic acid as D-calcium pantothenate; 33.1 mg of niacin; 551.3 mg of choline from choline chloride; 220.5 μg of biotin from D-biotin; and 1.65 mg of folic acid. An enzyme premix (Ronozyme B, Hoffmann La-Roche Inc., Nutley, NJ) provided per kilogram of diet: 70 U of endo-1,3(4)-β-glucanase (1 U = the amount of enzyme that releases 1.0 μ*M* of glucose or reducing carbohydrate/min at pH 5.0 and 30°C), 300 U of endo-1,4-β-xylanase (1 U = the amount of enzyme that releases 7.8 μ*M* of reducing xylose equivalents from azo-wheat arabinoxylan/min at pH 6.0 and 50°C), and 25 U of α-amylase (1 U = the amount of enzyme that breaks down 5.26 g of starch/h at pH 7.1 and 37°C).

[2]Monosodium phosphate contained 25.3% P, estimated at 100% available.

[3]Tylan provided 110 mg of tylosin/kg of diet (Elanco Global, Eli Lilly & Co., Greenfield, IN).

[4]Five trace mineral (TM) supplementation treatments were made to provide increasing concentrations of Fe, Zn, and Cu as $FeSO_4$, $ZnSO_4$, and $CuSO_4$, respectively, per kilogram of diet: TM0 = no supplemental Fe, Zn, or Cu; TM25 = 20 mg of Fe and Zn, and 2 mg of Cu; TM50 = 40 mg of Fe and Zn, and 4 mg of Cu; TM75 = 60 mg of Fe and Zn, and 6 mg of Cu; and TM100 = 80 mg of Fe and Zn, and 8 mg of Cu. All the TM treatments provided per kilogram of diet: 16 mg of Mn as $MnSO_4$, 0.15 mg of I as $Ca(IO_3)_2$, and 0.25 mg of Se as Na_2SeO_3. The 5 TM treatments and the 2 barley cultivars made a total 10 treatments in a factorial arrangement.

[5]Triplicate analyses of the barley cultivars, soybean meal, and the spray-dried whey provided the Ca, total P, phytic acid P, CP, and the AA values used to make the basal barley cultivar diets. Available P was calculated by subtracting phytate P from total P, and ME/kg was calculated using values from NRC (1998).

Apparent Zn and Cu Balance. Diets contained 0.05% Cr_2O_3 (Table 2) as an indigestible indicator. Fecal samples (about 100 g of DM without feed contamination) and total urine collections were made twice daily from d 22 to 26. Fecal samples were stored in plastic freezer bags. Urine was collected in plastic pails containing 30 mL of 6 N HCl. The total urine volume was recorded, and 10% was saved in 1-L, wide-mouthed, screw-capped, plastic bottles. Fecal and urine samples were immediately frozen at −20°C until analyzed. Each individual pig crate, fecal collection screen, and urine collection pail was washed immediately after each collection.

The 5-d fecal collections for individual barrows were thawed, pooled, mixed, and air-dried in an oven at 55°C for 48 h. The dried fecal samples and samples of each diet were ground to pass a 1.0-mm screen (Wiley mill, model No. 4, Arthur H. Thomas Co., Philadelphia, PA). The 5-d urine collections for individual barrows also were thawed, mixed, and subsampled for analysis. Triplicate samples of the diets and feces were digested using a wet ash procedure and analyzed for Zn, Cu, and Cr as described previously to determine Zn and Cu balance. Urine samples were also analyzed in triplicate for Zn and Cu. Iron balance was not determined because of the high dietary Fe content provided by the ingredients.

Statistical Analysis

All data were analyzed by ANOVA as a randomized complete block design with a 2 (barley cultivars) × 5 (TM treatments with increasing concentrations of Fe, Zn, and Cu) factorial arrangement that made a total of 10 treatments (Snedecor and Cochran, 1989) using the GLM procedure (SAS Inst. Inc., Cary, NC). Individual barrows were the experimental units. The model had 1 df for comparison of the barley cultivars, 4 df for the TM treatments, and 4 df for the barley cultivar × TM treatment interactions. Additional preplanned single df comparisons were the NB vs. the M955 barley cultivar for the slope and curvature of the response criteria lines (linear and quadratic) for the 5 TM treatments, and the linear and quadratic responses for the 5 TM treatments with both barley cultivars pooled. Significance was assumed at $P \leq 0.05$, and significant TM treatment criteria means were separated using the least significant difference test (Snedecor and Cochran, 1989).

RESULTS

There were no barley cultivar (NB vs. M955) × TM treatment interactions for any of the response criteria measured in this experiment, and the slope or curvature of the response criteria lines (linear or quadratic) for the TM treatments were not different for the NB vs. the M955 barley cultivars. Therefore, the main effect means are reported for the 2 barley cultivars and the 5 TM treatments (Tables 3 and 4).

Growth Performance, Hematocrit Volume, and Bone Characteristics

There were no differences between the NB and M955 barley cultivars for any of the response criteria measured (Table 3). However, for the TM treatments, there were linear increases ($P \leq 0.05$) in overall (0 to 28 d) ADFI and ADG, hematocrit volume on d 13 and 27, metacarpal fresh bone and ash weights, and metacarpal breaking strength with increasing concentration of TM (Fe, Zn, and Cu) treatment. Also, there were TM treatment responses ($P \leq 0.05$) for ADFI, ADG, hematocrit volume on d 13 and 27, and bone breaking strength because of the low values for pigs on TM0 compared with pigs on TM50, TM75, or TM100.

However, there were no TM treatment responses for metacarpal length and the midshaft widths at the widest and narrowest points, respectively, with overall experimental means (mm ± SE) of 53.44 ± 0.98, 14.57 ± 0.29, and 11.19 ± 0.24 (data not reported). Also, the concentrations of Zn and Cu in metacarpal bone ash did not differ among the TM treatments, with overall experimental means (mg/kg of bone ash ± SE) of 1,346 ± 113 and 6.0 ± 0.1, respectively.

Apparent Absorption, Retention, and Excretion of Zn and Cu

There were no differences between the NB and M955 cultivars for any of the Zn and Cu balance response criteria measured (Table 4). However, for the TM treatments, there were linear increases in the mg of Zn ($P < 0.001$) and Cu ($P \leq 0.01$) absorbed, retained, and excreted in feces and urine daily, and linear increases in the percentages of total (fecal + urinary) Zn and Cu excreted with increasing dietary concentration of TM treatment. Also, there were linear decreases ($P < 0.001$) in the percentages of Zn and Cu that were absorbed and retained with increasing concentration of TM treatment.

DISCUSSION

For the TM (Fe, Zn, and Cu) treatments in the current experiment, the linear increases in pig growth performance, hematocrit volume, metacarpal fresh bone and ash weight, metacarpal breaking strength, and the apparent absorption and retention (mg/d) of Zn and Cu with increasing concentration of TM treatment were expected because the TM0 to TM75 treatments were deficient in Fe and Zn, and the TM0 to TM50 treatments were deficient in Cu. Only the TM100 treatment provided 100% of the Fe and Zn requirements for swine from 10 to 20 kg of BW (NRC, 1998). These treatment responses are in agreement with other experiments in which baby pigs fed semi-purified diets (Shanklin et al., 1968; Hankins et al., 1985; Bobilya et al., 1991) and weanling pigs fed corn-SBM diets (Revy et al., 2006) had linear increases in response criteria that included

Table 3. Main effects of low-phytate barley and trace mineral (TM; Fe, Zn, and Cu) supplementation on growth performance, bone characteristics, and hematocrit volume of young barrows[1]

Item	Barley cultivar[2]			TM supplementation treatment[3]					
	Low-phytate mutant 955	Wild type normal	SEM	TM0	TM25	TM50	TM75	TM100	SEM
Barrows, No./mean	25	25		10	10	10	10	10	
Pig BW, kg									
d 0	9.94	9.93	0.18	9.95	9.94	9.92	9.92	9.96	0.28
d 28	23.57	23.70	0.65	21.30	23.25	24.49	24.21	24.95	1.00
ADG, overall,[4] g	486	491	20	405	475	520	510	535	31
ADFI, overall,[4] g	847	852	20	753	812	890	878	907	30
G:F, overall, g/kg	574	576	17	538	585	584	581	590	26
Metacarpal bone									
Fresh bone wt,[5] g	9.95	9.99	0.24	9.32	9.88	10.06	10.12	10.48	0.38
Ash wt,[5] g	1.61	1.63	0.04	1.50	1.58	1.63	1.68	1.70	0.07
Breaking strength,[4] kg	35.75	36.65	1.10	32.87	36.62	37.50	36.95	37.66	1.60
Hematocrit volume,%									
d 13[4]	42.70	43.13	0.99	38.92	43.15	44.15	43.70	44.65	1.55
d 27[4]	44.85	45.15	1.42	41.12	44.68	46.35	46.62	46.80	2.20

[1]There were no barley cultivar × TM treatment interactions, and the slopes of the lines for the TM treatment responses did not differ for low-phytate mutant barley vs. wild type barley. Therefore, main effect means are presented for the barley cultivar and the TM treatments.

[2]There were no barley cultivar (mutant 955 vs. wild type) main effect responses.

[3]The 5 TM supplementation treatments provided increasing concentrations of Fe, Zn, and Cu as sulfate salts per kilogram of diet: TM0 = No Fe, Zn, or Cu provided; TM25 = 20 mg of Fe and Zn, and 2 mg of Cu; TM50 = 40 mg of Fe and Zn, and 4 mg of Cu; TM75 = 60 mg of Fe and Zn, and 6 mg of Cu; and TM100 = 80 mg of Fe and Zn, and 8 Mg of Cu. All the TM treatments provided per kilogram of diet: 16 mg of Mn as $MnSO_4$; 0.15 mg of I as $Ca(IO_3)_2$; and 0.25 mg of Se as Na_2SeO_3.

[4]For the TM treatments, there were treatment responses ($P \leq 0.05$) because of the reduced values for TM0 vs. TM50, TM75, and TM100; and linear increases ($P \leq 0.05$) with increasing TM supplementation.

[5]For the TM treatments, there were linear increases ($P = 0.05$) with increasing TM supplementation.

growth performance, bone weight and strength, bone Zn concentration, and mg of Zn absorbed and retained with increasing dietary concentrations of Zn up to the Zn requirement.

In another experiment with growing swine, supplementation of a corn-SBM diet with increasing concentrations of Zn increased bone Zn concentrations, but had no effect on growth performance (Wedekind et al., 1994). Also, baby pigs fed a semi-purified test diet with increasing concentrations of Cu had increasing responses in growth, Cu balance, and tissue Cu concentrations up to the Cu requirement (Okonkwo et al., 1979).

Soybean meal was a major source of phytate in the basal diets in the current experiment and contributed about 0.10% phytate P (0.355% phytic acid) to the M955 and NB basal diets. Other experiments that evaluated LPA corn and barley mutants for young swine reported that the phytate in SBM had a significant negative effect on P utilization in practical grain-SBM diets (Veum et al., 2001, 2002, 2007). The phytate in soybean protein also has a strong negative effect on the availability and apparent absorption of Zn by chicks (O'Dell and Savage, 1960; Savage et al., 1964; Edwards and Baker, 2000) and swine (Oberleas et al., 1962; Bobilya et al., 1991; Lei et al., 1993). For chicks, the negative effect of phytate on trace mineral availability in soybeans and soy flour was greater for Zn than for Cu and Fe (Davis et al., 1962). Because of the negative effect of phytate in SBM on mineral availability, the development of LPA SBM (Wilcox et al., 2000; Hitz et al., 2002; Oltmans et al., 2005) for feeding in combination with LPA grains (Larson et al., 1998; Raboy et al., 2001; Dorsch et al., 2003) will greatly increase the utilization and reduce the excretion of minerals in practical diets for poultry and swine.

The TM treatment responses in the current experiment were affected by the pig body stores of Fe, Zn, and Cu at the start of the experiment; the availability (%) of the endogenous Fe, Zn, and Cu in the dietary ingredients (barley cultivars, SBM, and dried whey); the deficiency level (%) of the specific TM treatment fed; and the 28-d duration of the experiment. In another experiment where wet-autoclaved spray-dried egg white was the test protein used in semi-purified diets to determine the Zn requirement of neonatal pigs, there was no response in growth performance with increasing concentrations of Zn the first 14 d of the 28-d experiment (Hankins et al., 1985). In an experiment with late-finishing swine, the removal of all the inorganic P, Zn, Cu, and Mn from a corn-SBM diet (withdrawal diet) fed from 84 to 123 kg of BW (50 d) had no negative effects on growth performance or carcass characteristics, although metacarpal dry bone weight and ash weight were reduced in pigs fed the withdrawal diet compared with pigs fed the positive control diet (Peter et al., 2001). An experiment that evaluated the supplementation of practical nursery diets for weanling pigs with increasing concentrations of Fe also showed that the hematological criteria were not responsive to the Fe treatments until d 21 of the experiment, after which the hematological criteria increased linearly with increasing dietary Fe up to 100 mg/kg (Rincker et al., 2004).

Table 4. Effects of low-phytate barley and trace mineral (TM; Fe, Zn, and Cu) supplementation on the apparent absorption, retention, and excretion of zinc and copper[1]

Item	Barley cultivar[2] Low-phytate mutant 955	Barley cultivar[2] Wild type normal	SEM	TM supplementation treatment[3] TM0	TM25	TM50	TM75	TM100	SEM
Barrows, No./mean	25	25		10	10	10	10	10	
ADFI during collection, d 22 to 26,[4] kg	1.18	1.20	0.03	1.04	1.08	1.27	1.27	1.30	0.05
Zn									
Intake,[4] mg/d	85.3	85.7	1.8	29.8	52.8	87.7	113.9	143.2	2.8
Absorbed,[4] mg/d	11.2	10.9	0.6	7.9	9.5	10.6	12.9	14.1	1.0
Fecal,[4] mg/d	74.1	74.8	1.7	21.9	43.3	77.1	101.0	129.1	2.6
Urinary,[4] mg/d	1.3	1.1	0.1	0.7	0.8	1.3	1.4	1.8	0.2
Retained,[4] mg/d	9.9	9.8	0.6	7.2	8.7	9.3	11.5	12.3	1.0
Total excretion,[4] mg/d	75.4	75.9	1.7	22.6	44.1	78.4	102.4	130.9	2.6
Absorbed/intake,[5] %	13.1	12.7	0.9	26.5	18.0	12.1	11.3	9.8	1.4
Retained/intake,[5] %	11.6	11.4	0.9	24.2	16.5	10.6	10.1	8.6	1.4
Total excretion/intake,[4] %	88.4	88.6	0.9	75.8	83.5	89.4	89.9	91.4	1.4
Cu									
Intake,[4] mg/d	14.4	14.6	0.3	8.2	10.7	15.1	17.7	20.7	0.5
Absorbed,[6] mg/d	3.6	3.5	0.1	2.9	3.0	3.6	3.9	4.1	0.2
Fecal,[4] mg/d	10.8	11.1	0.3	5.3	7.7	11.5	13.8	16.6	0.4
Urinary,[6] mg/d	0.3	0.3	0.02	0.2	0.2	0.2	0.3	0.4	0.03
Retained,[6] mg/d	3.3	3.2	0.1	2.7	2.8	3.4	3.6	3.7	0.2
Total excretion,[4] mg/d	11.1	11.4	0.3	5.5	7.9	11.7	14.1	17.0	0.4
Absorbed/intake,[5] %	25.0	24.0	0.7	35.4	28.0	23.8	22.0	19.8	1.1
Retained/intake,[5] %	22.9	21.9	0.8	32.9	26.2	22.5	20.3	17.9	1.2
Total excretion/intake,[4] %	77.1	78.1	0.8	67.1	73.8	77.5	79.7	82.1	1.2

[1]There were no barley cultivar × TM treatment interactions, and the slopes of the lines for the TM treatment responses did not differ for low-phytate mutant barley vs. wild type barley. Therefore, main effect means are presented for the barley cultivar and the TM treatments.

[2]There were no barley cultivar (mutant 955 vs. wild type) main effect responses.

[3]The 5 TM supplementation treatments provided increasing concentrations of Fe, Zn, and Cu as sulfate salts per kilogram of diet: TM0 = No Fe, Zn, or Cu provided; TM25 = 20 mg of Fe and Zn, and 2 mg of Cu; TM50 = 40 mg of Fe and Zn, and 4 mg of Cu; TM75 = 60 mg of Fe and Zn, and 6 mg of Cu; and TM100 = 80 mg of Fe and Zn, and 8 mg of Cu. All the TM treatments provided per kilogram of diet: 16 mg of Mn as $MnSO_4$; 0.15 mg of I as $Ca(IO_3)_2$; and 0.25 mg of Se as Na_2SeO_3.

[4]For the TM treatments, there were linear ($P < 0.001$) increases with increasing TM supplementation.

[5]For the TM treatments, there were linear ($P < 0.001$) decreases with increasing TM supplementation.

[6]For the TM treatments, there were linear ($P \leq 0.01$) increases with increasing TM supplementation.

The lack of a LPA barley cultivar effect (M955 vs. NB) on the response criteria (growth performance, hematocrit volume, bone characteristics, and Zn and Cu balance) in the current experiment may be attributed to the small increases in the availability of the trace minerals in the practical diets containing M955 compared with NB. Based on Zn retention by young broiler chicks fed casein-based test diets without any added inorganic Zn, the apparent availability of Zn in the M955 and NB cultivars was determined to be about 63 and 43%, respectively, with a decline in Zn retention (%) as the inorganic Zn supplementation increased (Linares et al., 2007). Assuming that the availability (based on retention) of Zn in M955 and NB is about the same for young pigs as reported for young broiler chicks, the M955 and NB cultivars in the current experiment would contribute about 8.4 and 5.3 mg, respectively, of available Zn/kg of basal barley cultivar diet (treatment TM0) without any added inorganic Zn (from Tables 1 and 2, 52.75% M955 × 25.4 mg of Zn/kg × 63% = 8.4 mg/kg, and 51.98% NB × 23.8 mg of Zn/kg × 43% = 5.3 mg/kg). This calculation shows that the M955 barley would only provide about 3.1 mg more available Zn/kg of diet than the NB cultivar, and only 2.6 mg more available Zn/pig daily based on ADFI (Table 3). This small increase in available Zn as milligrams per kilogram of diet or milligrams per pig daily does not make a significant contribution to the available Zn requirement for young swine, which was determined to be between 26 and 31 mg/kg of phytate-free semi-purified diet for baby pigs (Hankins et al., 1985). In other experiments that evaluated the availability of trace minerals in cereal grains for growing and finishing swine, the availability of Zn and Cu were greater in barley than in wheat, and least in corn (Kalnitskii et al., 1986a,b).

The determination of the availability of Zn in SBM for young chicks was affected by the protein source used in the test diet (Edwards and Baker, 2000). When the test diet protein was soy protein concentrate compared with egg white, the availability of Zn in SBM was 78 or 40%, respectively, with 78% as the appropriate value to use for Zn availability in SBM with grain-SBM diets for poultry (Edwards and Baker, 2000). For the current experiment, assuming that the 60.4 mg of Zn/kg in SBM was 78% available, the SBM in both basal barley diets would provide about 11 mg of available Zn/kg of diet (23.5% SBM × 60.4 mg of Zn/kg of SBM × 78% = 11 mg).

The availability of minerals in diets fed to rainbow trout containing LPA barley cultivars compared with NB was evaluated in 2 experiments, and no differences were reported for apparent absorption (%) of Fe, Zn, or Cu by fish fed the diets containing the LPA cultivars or NB, although there was a significant increase in the apparent absorption of P in both experiments (Sugiura et al., 1999; Overturf et al., 2003). Overturf et al. (2003) suggested that sequestering of the trace minerals in the water tanks by the fish feces before the fecal collections were made once daily contributed to the negative apparent absorption coefficients for Fe and Zn in that experiment. Also, in another experiment, the apparent absorption of Zn, but not Cu, was increased in growing rats fed diets containing an LPA barley cultivar compared with a NB cultivar (Poulsen et al., 2001).

Other approaches that increased the availability of Zn in grain-SBM diets fed to young swine were the fermentation of the SBM with *Aspergillus usamii* before diet preparation (Matsui et al., 1998), or supplementation of the diet with microbial phytase (Lei et al., 1993). In other experiments, supplementation with microbial phytase increased the utilization of Zn and Cu, but not Mn, in grain-SBM diets fed to young pigs (Kirchgessner et al., 1994; Adeola et al., 1995; Windisch and Kirchgessner, 1996). In another experiment, microbial phytase did not increase the apparent absorption of Fe, Zn, Cu, or Mn in weanling pigs fed corn-SBM diets (Valencia and Chavez, 2002). For growing and finishing pigs, replacement of the Zn and Cu supplementation with phytase reduced the excretion of Zn and Cu without any negative effect on growth performance (Masuda et al., 2002). Based on literature data, microbial phytase added at 1,000 units/kg of diet was equivalent to the addition of 24 or 19 mg of Zn as $ZnSO_4$/kg of diet for pigs weighing 15 or 25 kg, respectively (Revy et al., 2003).

In the current experiment, the percentages of Zn and Cu that were absorbed and retained declined with increasing Zn and Cu supplementation of the barley cultivar diets, with corresponding increases in Zn and Cu excretion, which is in agreement with the responses reported for young broiler chicks fed diets made with these barley cultivars that had increasing concentrations of Zn supplementation (Linares et al., 2007). In the current experiment, the percentages of Zn and Cu that were absorbed, retained, and excreted are within the range of values reported for weanling pigs in other experiments (Carlson et al., 2004; Veum et al., 2004; Buff et al., 2005). The hematocrit volumes are also within the range of values reported for weanling pigs in other experiments (Kline et al., 1973; Dove and Haydon, 1991; Rincker et al., 2004).

In conclusion, replacing NB with the LPA barley cultivar M955 in the current experiment did not increase the utilization of Fe, Zn, and Cu in practical diets for young swine, apparently because of the small increase in the availability of the endogenous trace minerals in the diets containing M955 compared with NB. However, for the TM treatments, there were linear increases in growth performance, metacarpal bone ash and strength, hematocrit volume, and Zn and Cu absorption, retention, and excretion (mg/d) with increasing concentrations of Fe, Zn, and Cu provided as $FeSO_4$, $ZnSO_4$, and $CuSO_4$, with the greatest treatment concentration providing 100% of the NRC (1998) requirement for Fe and Zn, and 160% of the requirement for Cu. These results indicate that the inorganic trace mineral supplementation should not be less than the NRC (1998) requirements for young swine.

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